SYNTHESES, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF NEW HYDRAZINYL THIAZOLYL COUMARIN DERIVATIVES

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SYNTHESES, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF NEW HYDRAZINYL THIAZOLYL COUMARIN DERIVATIVES

by

AFSHEEN ARSHAD

Thesis submitted in fulfillment of the requirements

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Dedicated to my husband Wasim Sarwar

and my kids, Mahad Wasim, Muzammil Wasim

and Sabah Wasim

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

Solvents

EtOH	Ethanol
MeOH	Methanol
EtOAc	Ethyl acetate
$CHCl_3$	Chloroform
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated Dimethyl sulfoxide
$CDCl_3$	Deuterated chloroform
C_5D_5N	Deuterated pyridine

Chemicals

NH_4OH	Ammonium hydroxide
$DPPH^{\cdot}$	1,1-Diphenylpicrylhydrazyl radical
NA	Nutrient agar
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MTT	(3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide
BHT	Butylated hydroxytoluene

Instruments and Techniques

HRESIMS	High resolution electrospray mass spectrometry
IR	Infra red
^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon nuclear magnetic resonance
COSY	Correlated spectroscopy
HMQC	Heteronuclear multiple quantum coherence
HMBC	Heteronuclear multiple bond connectivity

TLC	Thin layer chromatography

Symbols

m/z	mass/charge
amu	atomic mass unit
mp	melting point
nm	nano meter
MHz	mega hertz
J	coupling constant
s	singlet
br s	broad singlet
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
t	triplet
td	triplet of doublets
q	quartet
quin	quintet
sex	sextet
m	multiplet
α	alpha
β	beta
µM	micro molar
µg	microgram
CFU	colony forming unit
1D	one dimensional
2D	two dimensional
ppm	parts per million
Bn	benzyl
RT	reverse transcriptase
ADP	adenosine diphosphate

NADPH	nicotinamide adenine dinucleotide phosphate

Microbes

S.aureus	*Staphylococcus aureus*
S. pyogenes	*Streptococcus pyogenes*
H. influenzae	*Haemophillus influenzae*
C.albicans	*Candida albicans*
M. tuberculosis	*Mycobacterium tuberculosis*

SINTESIS, PENCIRIAN DAN AKTIVITI BIOLOGI TERBITAN BARU KUMARIN HIDRAZINIL THIAZOLIL

ABSTRAK

Kumarin dan thiazol merupakan dua jenis farmakofor yang penting, yang mana sintesisnya diberikan keutamaan dalam pelbagai bidang kimia perubatan disebabkan kepelbagaian aktiviti farmakologi yang berkaitan dengan komponen gelang heterosiklik. Kajian ini bertujuan untuk mensintesis komponen (45) yang aktif daripada segi biologi, dengan menggabungkan gelang thiazol dan nukleus kumarin. Dua siri baru terbitan hidrazinil thiazolil kumarin telah di sintesis dengan empat langkah. Langkah pertama ialah penyediaan sebatian asetil kumarin kondensasi Knoevenagel yang melibatkan penukargantian salisaldehid dengan etil asetoasetat dengan kehadiran piperidina sebagai mangkin. Dalam langkah kedua, pembrominan asetil kumarin dengan kehadiran bromin, dilakukan untuk mendapatkan bromoasetil kumarin. Thiosemikarbazon dihasilkan dalam langkah ketiga yang mana thiosemikarbazid dioleh dengan pelbagai aldehid dan keton tertukarganti dengan menggunakan asid asetik glasial sebagai mangkin. Langkah sintesis terakhir melibatkan sintesis dua siri baru hydrazinil thiazolil kumarin menenisi pensiklikan Hantzch. Salah satu siri sebatian diperoleh daripada tindak balas antara 3-bromoasetil kumarin dengan thiosemikarbazon yang mempakan terbitan aldehid, tertukanganti manakala siri yang lain didapati daripada tindak balas antara terbitan ketone tertukanganti thiosemikarbazon dengan 3-bromoasetil kumarin. Ke semua sebatian dicirikan dengan menggunkan IR, ^{1}H NMR, ^{13}C NMR, elemental

analisis dan spektroskopi jisim. Struktur beberapa sebatian telah dikenalpasti dengan kristalografi sinar X dan 2D NMR (COSY, HMQC, HMBC).

Semua sebatian telah disaring secara *in vitro* bagi mengesan aktiviti antimikrobial terhadap spesis bakteria Gram-positif dan Gram-negatif termasuk *Mycobacterium tuberculosis* dan *Candida albicans*. Aktiviti antimikrobial yang ketara telah ditunjukkan terhadap *Mycobacterium tuberculosis* oleh sebatian **70i** dengan nilai MIC yang terendah iatu 2.6 µM. Di samping itu, sebatian **70e**, **71b**, **71e**, **74a**, **74b** dan **75b** menunjukkan aktiviti antimikrobial yang amat baik terhadap kebanyakan mikrob yang diuji.

Semua terbitan kumarin dikaji selanjutnya untuk mengetahui aktiviti antioksidan dengan menggunakan ujian radikal DPPH. Keputusan kajian menunjukkan bahawa ke semua sebatian yang disintesis (**70a–76b**) mempunyi aktiviti antioksidan yang tinggi, manakala sebahagian sebatian terbitan (**70a–70i**, **73b**, **74b** dan **75b–75f**) pula menunjukkan penyingkiran aktiviti yang lebih tinggi berbanding BHT (antioksidan sintetik) tetapi setanding dengan quercetin (antioksidan semulajadi) yang kedua-duanya mempakan rujukan piawai.

SYNTHESES, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF NEW HYDRAZINYL THIAZOLYL COUMARIN DERIVATIVES

ABSTRACT

Coumarin and thiazole are two important pharmacophores, the syntheses of which acquire great importance in medicinal chemistry due to the diverse pharmacological activities. The present study aims to synthesize some (45) new biologically active compounds by incorporating thiazole ring with coumarin nucleus. Two new series of hydrazinyl thiazolyl coumarin derivatives have been synthesized in four steps. The first step of the synthesis was the preparation of acetyl coumarins by Knoevenagel's condensation of substituted salicyaldehydes with ethyl acetoacetate in the presence of catalytic amount of piperidine. In the second step, bromination of acetyl coumarins was carried out with bromine in order to yield the corresponding bromoacetyl coumarins. Thiosemicarbazones were synthesized in the third step by treating thiosemicarbazide with various substituted aldehydes and ketones in the presence of a catalytic amount of glacial acetic acid. The final step of the synthesis involved the preparation of two new series of hydrazinyl thiazolyl coumarins using Hantzsch cyclization protocol. One series of compounds was obtained by reacting 3-bromoacetyl coumarins with thiosemicarbazones derived from various substituted aldehydes, while the other series was furnished by treating thiosemicarbazones of substituted ketones with 3-bromoacetylcoumarins. All the synthesized compounds were fully characterized by IR, ^{1}H and ^{13}C NMR, elemental analysis and mass spectroscopy. The structures of some

compounds were further confirmed by X-ray crystallography as well as 2D NMR (COSY, HMQC, HMBC) spectroscopic techniques.

All of these derivatives were screened *in vitro* for their antimicrobial activities against various Gram-positive and Gram-negative bacteria species including *Mycobacterium tuberculosis* and *Candida albicans.* Significant activity against *Mycobacterium tuberculosis* was shown by compound **70i** with the lowest MIC value of 2.6 µM. Moreover, compounds **70e**, **71b**, **71e**, **74a**, **74b** and **75b** exhibited very good antimicrobial activities against almost all of the tested microbial strains.

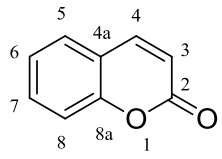
All synthesized hydrazinyl thiazolyl coumarin derivatives were further evaluated for their antioxidant activity by using $DPPH^{\cdot}$ radical assay. The results indicated that all the synthesized compounds (**70a–76b**) exhibited very good antioxidant activity, while some of the derivatives (**70a–70i**, **73b**, **74b** and **75b–75f**) showing radical scavenging activity even better than that of BHT (a synthetic antioxidant) but comparable to that of quercetin and catechin (natural antioxidants), the reference standards.

CHAPTER 1

INTRODUCTION

1.1. Coumarins

Coumarins constitute an important class of heterocyclic compounds found throughout the plant kingdom. The pharmacological and biochemical activities of coumarin derivatives encompass an important position in heterocyclic chemistry and in medicinal chemistry. The term coumarin was originated from 'coumarou', the French vernacular name for Tonka beans (*Dipteryx odorata*), from which the coumarin was first isolated by Vogel in 1820 (Bruneton, 1999). Coumarin belongs to the benzopyrone class of the compounds and its parent molecule (1,2-benzopyrone) is composed of the fused benzene and α-pyrone rings (**1**).



1

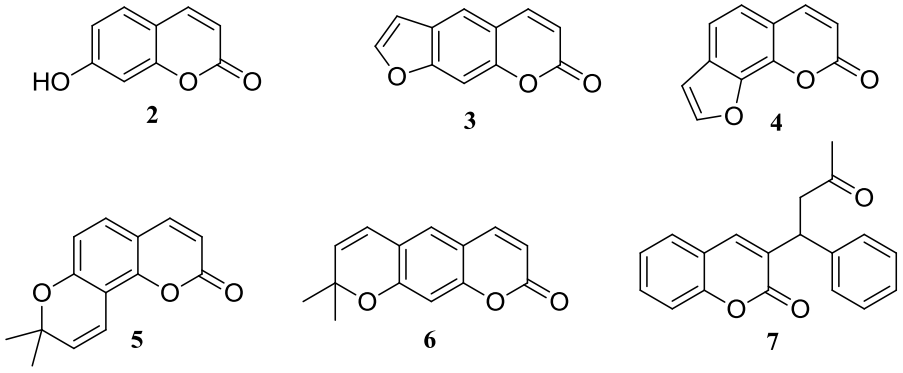
The coumarin derivatives are usually found as secondary metabolites in seeds, roots and leaves of higher plants but are found rarely in fungi and bacteria (Murray *et al.*, 1982). They play a vital role in plant biochemistry and physiology by acting as antioxidants, enzyme inhibitors and precursors of toxic substances. Moreover, coumarins are also involved in the control of respiration, photosynthesis and in the defence mechanism of plants against infections. Coumarins can be classified on the basis of various types of substitutions in the benzene or pyrone rings that influence their therapeutic values (Keating *et al.*, 1997). There are four main coumarin sub-groups:

i. Simple coumarins are hydroxylated, alkoxylated and alkylated derivatives of the parent molecule, such as 7-hydroxycoumarin (**2**).

ii. Furanocoumarins are based on five-membered furan ring attached to the coumarin nucleus either linearly or angularly. Psoralen (**3**) and angelicin (**4**) are good examples of furanocoumarins.

iii. Pyranocoumarins are similar to furanocoumarins but contain six-membered pyran ring, for example seselin (**5**) and xanthyletin (**6**).

iv. Pyrone-substituted coumarins have substitution on pyrone ring, usually at C-3 or C-4 position. The synthetic compound warfarin (**7**) belongs to this type of coumarin.



Diverse biological activities associated with the coumarin nucleus have drawn a considerable attention of synthetic chemists in recent years. The synthesis of coumarin derivatives can be carried out by various synthetic procedures, which include Pechmann reaction, Perkin reaction, Reformatsky reaction and Knoevenagel reaction. Koevenagel reaction was used to synthesize the novel coumarin derivatives in this study. It is a simple, fast and general procedure to synthesize 3-acetylcoumarins, where the condensation of salicylaldehyde or its derivative with ethyl acetoacetate leads to

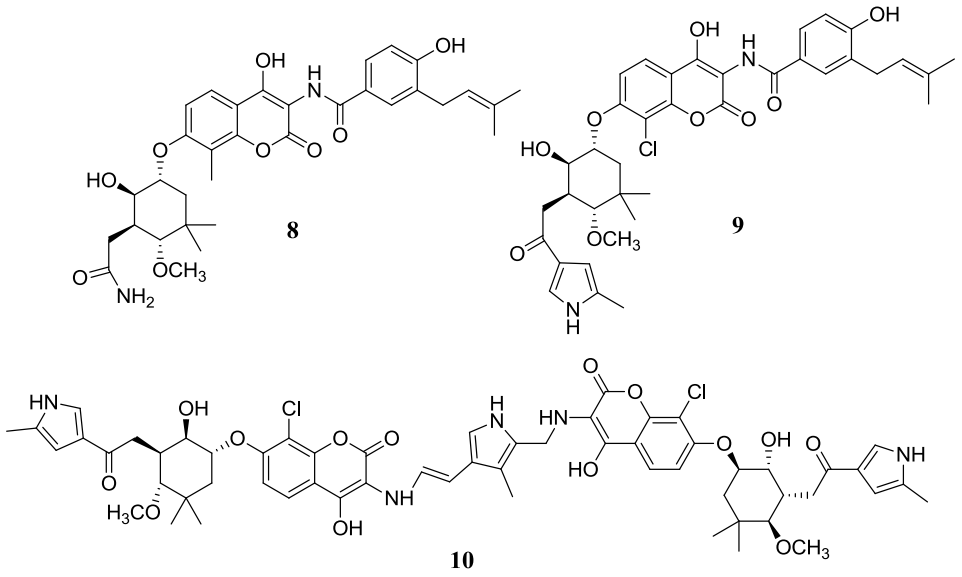
coumarin synthesis in the presence of piperidine as a catalyst. The reaction proceeds without any solvent. The solvent-free conditions have many advantages as solvents are often expensive, toxic, difficulty to remove solvents with high boiling points, and are environment polluting agents. Moreover, the work-up and purification procedures are simplified by avoiding liquid-liquid extraction for the isolation of reaction products (Bogda 1998).

1.2. Biological activities of coumarins

The natural and synthetic coumarin derivatives possess remarkable activities against bacteria, fungi, viruses and tumours. They also act as anti-coagulants, free radical scavengers, analgesics and anti-inflammatory agents. The physiological, bacteriostatic and anti-tumour activities of coumarins encourage the derivatization and screening of their analogues as new therapeutic agents.

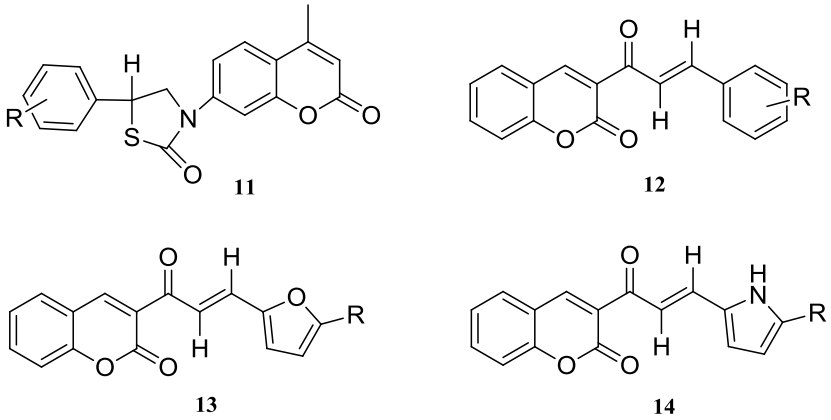
1.2.1. Antimicrobial activities of coumarins

Coumarin derivatives with diverse structures are reported to have remarkable activities against different strains of bacteria, fungi and viruses. Antibiotics of coumarin group such as novobiocin (**8**), clorobiocin (**9**) and coumermycin A_1 (**10**) are potent inhibitors of DNA gyrase (Hooper *et al.*, 1982). All these antibiotics have been isolated from different species of *Streptomyces* and contain 3-amino-4-hydroxycoumarin moiety and a substituted deoxysugar as the important parts of their structures, which are responsible for their biological activities (Chen and Walsh, 2001).



8 **9** **10**

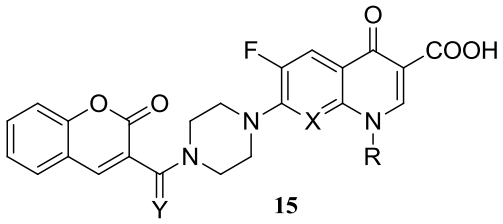
Besides naturally occurring coumarin antibiotics, several attempts were made to synthesize the coumarin-based antimicrobial compounds. Ronad and co-workers (2010) synthesized a series of 7-(2-substitutedphenylthiazolidinyl)-benzopyran-2-one derivatives (**11**) and evaluated the synthesized compounds against various bacterial and fungal strains. The results indicated that *para*-substituted nitro, methoxy and fluoro derivatives exhibited very good antibacterial and antifungal activities at a concentration of 100 µg/mL.



11 **12** **13** **14**

A subsequent study (Ajani and Nwinyi, 2010) reported the synthesis and antimicrobial activity of 3-acetylcoumarin derivatives, derived from its condensation with aromatic and heteroaromatic aldehydes to afford the corresponding aromatic chalcones (**12**) and heteroaromatic chalcones (**13**, **14**) respectively. The antibacterial activity of these compounds was tested by using three Gram-positive and two Gram-negative strains of bacteria. The MIC values of all the synthesized compounds were reported within the range of 7.8-31.2 µg/mL.

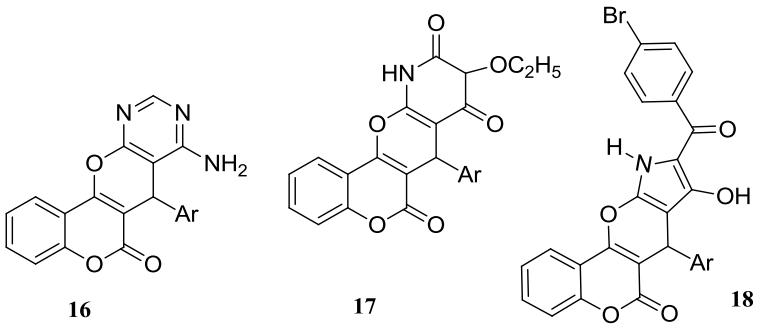
Moreover, Emami *et al.* (2008) reported a new series of quinolone-based coumarin derivatives with noteworthy antibacterial activities against various strains of *Staphylococcus* and *Bacillus*. The results indicated that *N*-[2-(coumarin-3-yl)ethyl] piperazinyl quinolone derivatives (**15**) exhibited equivalent or more potent antibacterial activities against all the tested strains as compared to that of the parent quinolones (norfloxacin, ciprofloxacin and enoxacin).



15

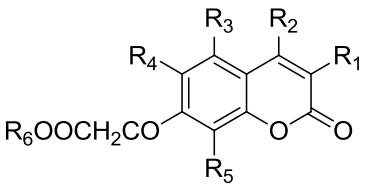
Another library of pyrimidino (**16**), pyridino (**17**) and pyrrolo (**18**) benzopyran derivatives containing heterocyclic rings fused with a coumarin moiety was synthesized by a group of researchers (Al-Haiza *et al.*, 2003) and they found that some of the synthesized compounds showed antibacterial activities against Gram-negative bacteria comparable to that of amoxicillin, the reference standard. Moreover, some of these

derivatives showed excellent activity against *Aspergillus niger*, which appeared to be even higher than the reference drug, mycostatin.



One of the most pathogenic bacterial strains is the *Mycobacterium tuberculosis*, which is responsible for most of the tuberculosis cases. Almost one-third of the world's population is infected by this pathogen and 1.7 millions deaths were attributed to this microorganism in 2009. This problem has now become more severe due to the resistance developed by *Mycobacterium tuberculosis* against the first line as well as second line drugs. Multidrug-resistant TB cases reported in 2010 were to be their highest level (Koul *et al.*, 2011). Hence, there is a need to develop new molecules against this deadly pathogen. Compounds having benzopyran core with several substitution patterns comprise a group exhibited remarkable activity against *Mycobacterium* (Ananthan *et al.*, 2009). One of the series of this group of compounds has variously substituted acetic acid ester moieties attached through an oxygen atom to C-7 position of the coumarin core (**19**). This series consisted of 87 compounds, 31% (27 compounds) of which showed inhibition more than 90% in the initial screening. The most potent compounds with TB IC_{90} values of 0.2-2.0 µg/mL were found to be those with substitution at C-4 along with the acetic acid moiety at C-7 position of the coumarin ring. The other analogous series of this group has a distinctive substitution

pattern, in which only one position of the coumarin ring was substituted with a carboxamide group at C-3 (**20**). This series consisted of 25 compounds and seven of which demonstrated more than 90% inhibition of *M. tuberculosis* in the primary assay.



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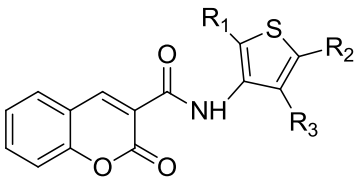
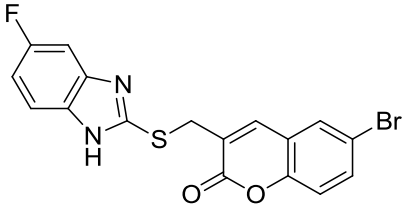


20

Coumarin derivatives are also potential antiviral drugs. Some new benzimidazole-coumarin conjugates were studied as anti-hepatitis C virus agents (Hwu *et al.*, 2008). The two moieties were linked by methylenethio linkage. Among the nineteen new conjugated compounds, 2-[(6′-bromocoumarin-3′-yl)methylenethio]-5-fluorobenzimidazole (**21**) and its derivative 1-[(2″,3″,4″,6″-tetra-*O*-acetyl)glucopyranose-1″-yl]-2-[(6′-bromocoumarin-3′-yl)methylenethio] (**22**) showed EC_{50} values of 3.4 and 4.1 µM respectively against the hepatitis C virus.



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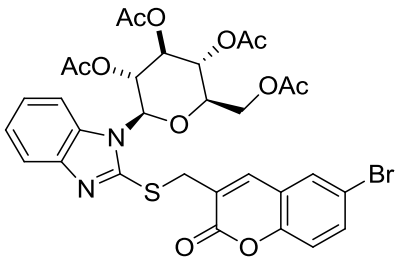
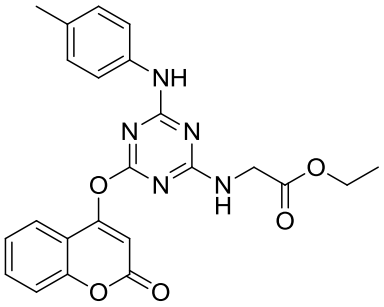


22

Moreover, Dharmesh *et al*. (2009) reported three novel series of 2-(coumarin-4-yloxy)-4,6-(disubstituted)-s-triazine derivatives as novel non-nucleoside reverse transcriptase inhibitors (NNRTIs) and their activities were assessed against human immunodeficiency virus HIV-1 (III-B), HIV-2 (ROD) and the double RT mutant HIV-1

(K103N and Y181C). The study established that substitutions at positions 4 and 6 of the coumarinyl-triazine scaffold showed moderate to good anti-HIV activity against tested HIV-strains compared to those of nevirapine and efavirenz, the reference standards. Compound **23** exhibited the highest activity with IC_{50} value of 1.07 µg/mL against HIV-1. In addition, the IC_{50} values of other *para*-substituted derivatives were within the range of 6.86-67.70 µg/mL (Mahajan *et al.*, 2009).



23

1.2.2. Antioxidant activity of coumarins

Antioxidants can be defined as substances, which in relatively low concentrations as compared to the oxidizable substrates significantly delay or prevent the oxidation process (Halliwell, 1994). The continuous metabolic reactions in a living system produced reactive oxygen species, such as hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$) and superoxide ($O_2^{\cdot}$)$^{-}$radicals. These free radicals are highly reactive and react readily with biomolecules and readily induce the chain reaction of free radicals formation (Halliwell and Gutteridge, 1990). The free radical induced oxidation of biomolecules is the major cause of various diseases including atherosclerosis, cancer, inflammation and arthritis (Aruoma, 1998). There is always a need to explore new antioxidants that can stop the

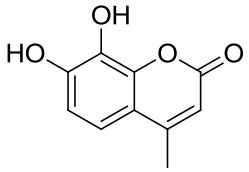
oxidative damages caused by the free radicals chain reaction by neutralizing them into the diamagnetic molecules.

Various coumarin derivatives are reported to exhibit the tissue-protective antioxidant property by acting as scavengers of free radicals. The antioxidant property of coumarins can be attributed to their structural similarities with flavonoids and benzophenones (Beillerot *et al.,* 2008). Most of the hydroxy coumarins are potent $H^{\cdot}$ donors and after donation they exist in the radical form. The radical forms of these derivatives are stabilized by electron delocalization across the highly conjugated molecular skeleton and thus prevented it to take part in oxidative reactions (Sharma *et al.,* 2005).

In vitro studies of some natural and synthetic coumarin dihydroxy derivatives revealed that *ortho*-dihydroxy coumarins were more effective than *meta*-substituted derivatives as lipid peroxidation inhibitors (Paya *et al.,* 1992). A subsequent study was conducted on the structure-activity relationship of some dihydroxy-4-methyl coumarins (DHMC) and their ternary (1:1:1) complexes with Fe (III) and ADP. The antioxidant capacity of all the synthesized compounds was determined by their superoxide radical $(O_2^{\cdot})^-$ scavenging activities. the The highest radical scavenging activity was shown by 7,8-dihydroxy,4-methyl derivative (**24**) and its complex with Fe (III)-ADP having IC_{50} values of 34 and 10.5 µM respectively. The results of structure-activity relationship suggested that the introduction of hydroxyl groups at C-7 and C-8 position of coumarin increases the antioxidant ability considerably (Sharma *et al.,* 2005).

Vukovic *et al.* (2010) reported a series of imino and amino derivatives of 4-hydroxycoumarins with antioxidant activity. This study revealed that amino derivatives exhibited higher scavenging activity as compared to that of the imino

analogues. The *para*-nitro phenyl derivatives of amino series (**25**) exhibited the most potent activity with IC_{50} value of 25.9 µM that was comparable to that of BHT, the reference standard. The results could be attributed to the presence of the amino group, which enhances the activity along with the hydroxyl group. Moreover, the intra-molecular hydrogen bonding in these molecules contributes additionally to increase the acidity of protons which in turn can be donated easily to the DPPH radical.



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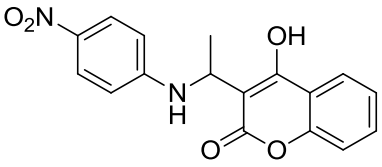
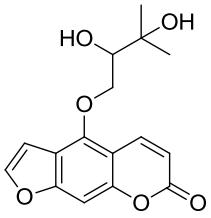


25

A related study (Chen *et al.*, 2006) was also conducted on the antioxidant potential of *ortho*-hydroxy amino derivatives of coumarins. This study demonstrated that coumarin derivatives with *ortho*-hydroxy-amino group exhibited even more potent antioxidant activities than that of the monohydroxy or dihydroxy derivatives of coumarins. This study suggested that *ortho*-hydroxy-amino group can be used as a potential functional group to synthesize novel antioxidants. The results of this study were further supported by the findings of Tyagi *et al.* (2005), who synthesized six novel 4-methylcoumarins with different functional groups, such as amino, hydroxyl, *N*-acetyl, acetoxy and nitro and examined their *in vitro* effects on NADPH dependant liver microsomal lipid peroxidation. They concluded that the amino group can be an effective substitute for hydroxyl group as it caused a dramatic inhibition of lipid peroxidation.

Besides these, antioxidant properties of furanocoumarins (**26**) (Piao *et al.*, 2004), bis and tetrakis-4-hydroxycoumarins (**27**, **28**) are also documented (Hamdi *et al.*, 2008).



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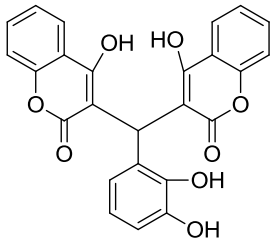

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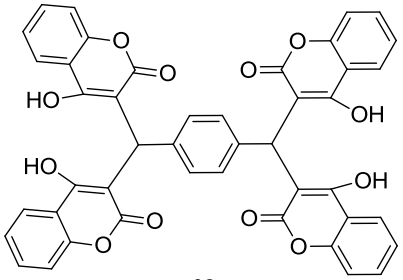

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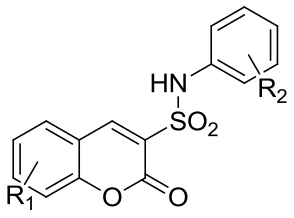
1.2.3. Anti-cancer activity of coumarins

Anti-cancer drugs are usually designed to damage the abnormally dividing cells by interrupting the process of cell division. Such drugs include DNA intercalating agents, DNA cross-linking agents, cytoskeleton-disrupting agents and anti-metabolites. Though, these drugs are effective, they exhibit severe side effects on normal proliferating tissues. However, literature reported several studies on the anti-proliferative and anti-tumour activities of various coumarin and its derivatives, which are not only beneficial for the treatment of cancer, but also used to treat the side effects of radiotherapy and chemotherapy (Grotz *et al.*, 2001). 7-Hydroxycoumarin, for example was reported to be a highly effective compound which inhibited the prostate cancer, malignant melanoma and metastatic renal cell carcinoma in clinical trials (Thornes *et al.*, 1994, Marshall *et al.*, 1991). The *in vitro* structure-activity relationship studies of coumarin derivatives can be related to the presence and the positions of

hydroxyl groups in their structures. It was found that cytotoxic activity was enhanced in the derivatives containing *ortho*-dihydroxy substituents (Kolodziej *et al.*, 1997).

Reddy *et al.* (2004) reported the syntheses of coumarin-3-(*N*-aryl)sulphonamides (**29**) via Knoevenagel condensation reaction of anilinosulfonylacetic acids with substituted salicyaldehydes and these derivatives were evaluated for their anticancer activities in various cancer cell lines. The androgen receptor negative prostate (DU145), colorectal (DLD-1), non-small cell lung carcinoma (H157), estrogen receptor negative breast (BT20) and chronic myeloid leukemia (K562) cell lines were used in this study. This study concluded that all the tested compounds have GI_{50} values less than 100 µM against different cancer cell lines and encourage the synthesis of more potent sulphonamide coumarin derivatives.



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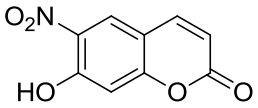

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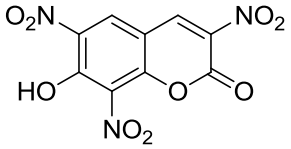

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In addition, a library of compounds including the hydroxylated and nitrated derivatives of coumarins was evaluated for their cytotoxic activities by using MTT assay against human skin melignant melanocytes (SK-MEL-31) and normal human skin fibroblastic cells (HS613.SK) in order to verify the selective toxicity of the coumarin derivatives. The whole series of the hydroxylated coumarins was found to be more toxic in HS613.SK than SK-MEL-31, whereas the novel synthetic nitrated coumarins, 6-nitro,7-hydroxycoumarin (**30**) and 3,6,8-trinitro,7-hydroxycoumarin (**31**) were proven to be significantly more toxic to SK-MEL-31 than HS613.SK cells. The results indicated that

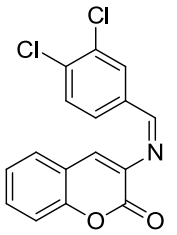
the nitro derivatives of coumarins can be explored as potential chemotherapeutic agents (Finn *et al.*, 2001).

In addition, coumarin and its derivatives are known to be effective in renal cell carcinoma (Kokron *et al.*, 1991), prostate cancer (Mohler *et al.*, 1992) and in leukaemia treatments (Wang *et al.*, 2002).

1.2.4. Anti-inflammatory and analgesic activities of coumarins

Several studies have been conducted to explore the anti-inflammatory and analgesic properties of coumarins. Maddi and his co-workers (1992) had investigated the anti-inflammatory activity of a series of synthetic 3-(benzylideneamino)coumarins and found that the 3,4-dichloro analogue (**32**) inhibited 67% of swelling in rats undergoing the carrageenan-induced paw oedema test in three hours. The same compound also showed analgesic activity similar to that of aspirin in the acetic acid-induced writhing test conducted in mice.

A novel series of 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines was synthesized by treating various substituted 3-aryl-1-(3-coumarinyl)propan-1-ones with phenylhydrazine in the presence of pyridine. All the synthesized derivatives were screened *in vivo* for their anti-inflammatory and analgesic activities at a dose of 200 mg/Kg. Among the twelve synthesized compounds, only compounds **33** and **34** showed remarkable anti-inflammatory activities in carrageenan-induced paw oedema and in adjuvant-induced arthritis in rats. Moreover, these compounds displayed significant analgesic and antipyretic activities with minimum ulcerogenic effect (Khode *et al.*, 2009).



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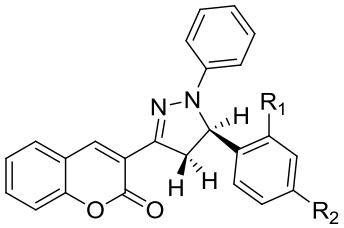
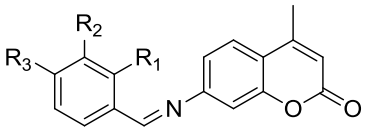


33 R_1 = Cl, R_2 = Cl
34 R_1 = H , R_2 = Cl



35 R_1 = Cl, R_2 = H, R_3 = Cl
36 R_1 = Cl, R_2 = H, R_3 = H
37 R_1 = H, R_2 = Br, R_3 =H

A subsequent study also reported the exceptional analgesic and anti-inflammatory effects of Schiff bases derived from 7-amino-4-methylcoumarin. In particular, compounds **35**, **36** and **37** exhibited more potent activities as compared to those of the reference drugs (Ronad *et al.*, 2008).

1.2.5. Miscellaneous activities of coumarins

Anti-coagulant activity of coumarins is well known. Warfarin, a synthetic derivative of dicoumarol is a popular anticoagulant drug. Lee *et al.* (2003) in a bio-assay guided isolation of methanol extract of roots of *Angelica genuflexa* and *A. gigas* (having a potent anti-thrombotic potential), isolated eight pure compounds of coumarin derivatives with noteworthy anti-coagulant and anti-platelet activities. Another study revealed that the derivatives of 4-methyl-7-hydroxycoumarin can be used as effective bio-pesticides against the larvae of *Aedes aegypti* and *Culex quinquefasciatus* (Deshmukh *et al.*, 2008). Moreover, coumarin and its derivatives are known as tyrosinase (Fais *et al.*, 2009), α-glucosidase (Raju *et al.*, 2010) and 5-lipoxygenase inhibitors (Grimm *et al.*, 2006).

Besides the aforementioned biological activities, coumarin derivatives also possess spectral, luminescent and lasing properties (Samsonova *et al.*, 2007). Amino coumarins,

for instance, are used extensively as active media in dye lasers in the blue-green range (Schimitschek *et al.*, 1976, Jones, 1980). In addition, pyrrole bis-coumarins are used as fluorescent probes (Shastri *et al.*, 2007).

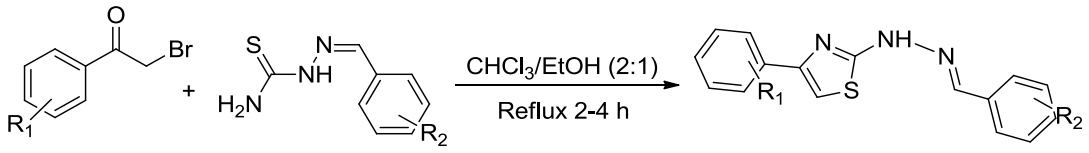
1.3. Thiazolyl coumarins

Thaizolyl coumarins are coumarin derivatives, in which one of the coumarin protons (usually at C-3) is substituted by a thiazole ring. Amino thiazole group is known as an important pharmacophore in medicinal chemistry. Compounds containing thiazole rings have remarkable medicinal values due to their potential chemotherapeutic (Liebig *et al.*, 1974), fungicidal (Pathak *et al.*, 1981), antiviral (Spector *et al.*, 1998) and pesticidal (Fan *et al.*, 2006) properties. In addition, 2-aminothiazole derivatives are reported to exhibit significant biological activities such as anti-tuberculosis (Sytnik, 2003), anti-inflammatory (Holla *et al.*, 2003), enzyme inhibition (Das *et al.*, 2006) and anti-tumour activities (El-Subbagh *et al.*, 1999). They have also found broad applications in the treatment of allergies (Shigenaga *et al.*, 1993), schizophrenia (Ohkubo *et al.*, 1995) and hypertension (Patt *et al.*, 1992).

1.3.1. Methods for the synthesis of thiazoles

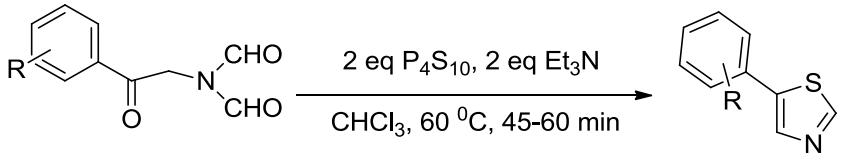
Several methods were reported in the literature for introducing the thiazole ring in the molecular skeleton. Hantzch synthesis, which was first introduced by a German chemist Hantzsch in 1887, is the most valuable and versatile among all thiazole syntheses. This method involved the condensation of a compound having two hetero atoms on the same carbon with a carbonyl compound bearing one halogen atom on the adjacent carbon

(**Scheme 1.1**). In this reaction, a variety of compounds may serve as nucleophilic reagents, such as thiourea, thioamide, thiosemicarbazide, ammonium thiocarbamate or dithiocarbamate and their derivatives (Joule *et al.*, 1995).



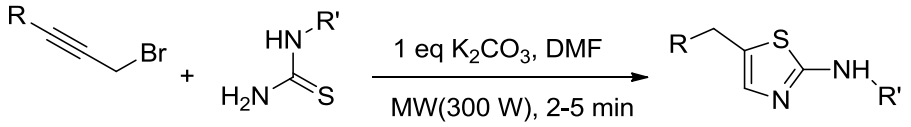
Scheme 1.1: Synthesis of thiazoles by Hantzch reaction

Gabriel's synthesis (**Scheme 1.2**) is also used to synthesize arylthiazoles in good yield by the treatment of *N,N*-diformylaminomethyl aryl ketones with phosphorous pentasulphide in triethylamine and chloroform (Sheldrake *et al.*, 2006).



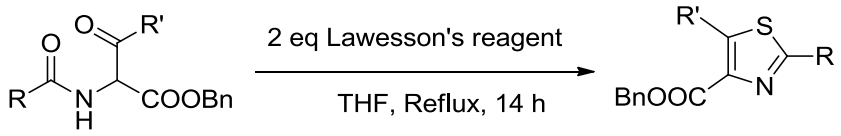
Scheme 1.2: Synthesis of thiazoles by Gabriel's synthesis

A single pot alkylation-cyclization reaction of propargyl bromides with thiourea afforded 2-aminothiazoles under microwave irradiation in few minutes as shown in (**Scheme 1.3**) (Castagnolo *et al.*, 2009).



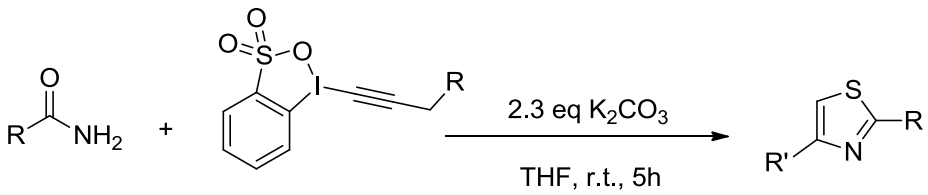
Scheme 1.3: Synthesis of thiazoles by alkylation-cyclization reaction

Thiazole scaffolds with structural diversity in positions two and five can be synthesized by cyclization of *α*-amido-*β*-ketoesters by reacting with Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione) as explained in **Scheme 1.4** (Sanz-Cervera *et al.*, 2009).



Scheme 1.4: Synthesis of thiazoles by cyclization of *α*-amido-*β*-ketoesters

Thiazoles can be obtained in good yields by the reaction of *1H*-1-(1´alkynyl)-5-methyl-1,2,3-benzoiodoxathiole,3,3-dioxides with thioamides (**Scheme 1.5)**. The side product potassium 2-iodo-5-methylbenzenesulfonate can be recovered easily and is used for the regeneration of *1H*-1-(1´alkynyl)-5-methyl-1,2,3-benzoiodoxathiole,3,3-dioxides quantitatively (Ishiwata and Togo, 2008).

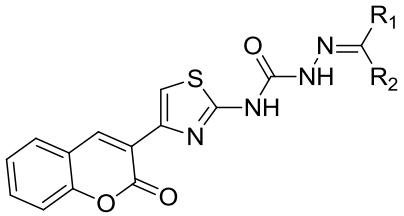


Scheme 1.5: Synthesis of thiazoles by the reaction of *1H*-1-(1´alkynyl)-5-methyl-1,2,3-benzoiodoxathiole,3,3-dioxides with thioamides

In the present study, Hantzsch cyclization was used to introduce thiazole ring into the molecular framework. This method has several advantages with wide scope. It allows alkyl, aryl or heterocyclic substituents to be place in any position of the 5-membered ring. It involves simple work-up and usually the result is a single product.

1.3.2. Biological activities of thiazolyl coumarins

Coumarin derivatives having various substituted thiazole rings at carbon-3 were reported to exhibit promising biological activities (Kumar *et al.*, 2008). Siddiqui and co-workers (2009) reported the syntheses of some new coumarin-incorporated thiazolyl semicarbazones (**38**) with good anti-convulsant activity, while analgesic and anti-inflammatory activities of coumarin thiazolyl quinolones (**39**) (Kalkhambkar *et al.*, 2007) are also known.



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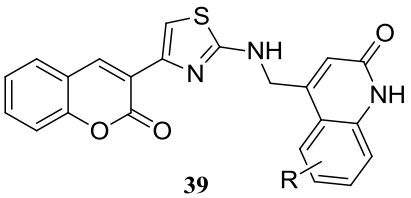

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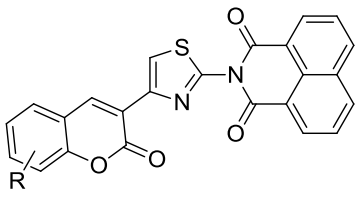
1.3.2.1. Antimicrobial activity of thiazolyl coumarins

The synthesis of thiazolyl coumarins is attracting great interests of medicinal chemists due to their wide range of pharmacological activities, especially remarkable antimicrobial activity with broad spectrum. Kamal *et al.* (2009) synthesized a series of coumarin-naphthalimide conjugates (**40**) linked through the thiazolyl ring by reacting aminothiazolyl coumarins with naphthalic anhydride. All the synthesized conjugates were assayed against different strains of Gram-positive and Gram-negative bacteria as well as few strains of fungi. In addition, anti-cancer activity was also evaluated against colon, breast and lung cancer cell lines. Some of the conjugates showed equipotent growth inhibition as compared to those of the reference drugs and hence regarded as potential anticancer and antimicrobial agents.



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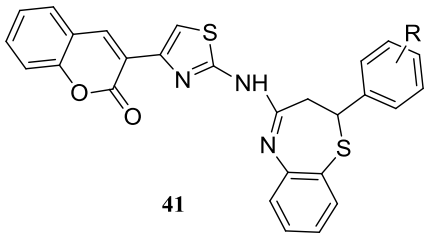

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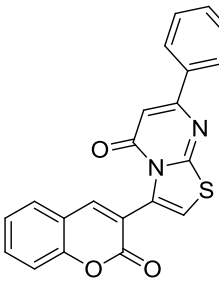
In another study, antibacterial and antifungal activities of 2,3-dihydro-2-aryl-4-[4-(2-oxo-*2H*-chromen-3-yl)-1,3-thiazol-2-ylamino]-1,5-benzothiazepines (**41**) against a variety of bacterial strains and some fungal pathogens were investigated (Raval *et al.*, 2008).

Furthermore, a group of researchers assayed *in vitro* antimicrobial activities of coumarinyl thiazolopyrimidinones (**42**) and these compounds were found to be specifically active against Gram-positive bacteria but were incapable to inhibit the growth of Gram-negative bacterial strains. However, moderate fungal activity against *Candida albicans* and *Penicillium chrysogenum* was observed but most of the compounds were inactive against *Aspergillus niger* (Yaragatti *et al*., 2010).



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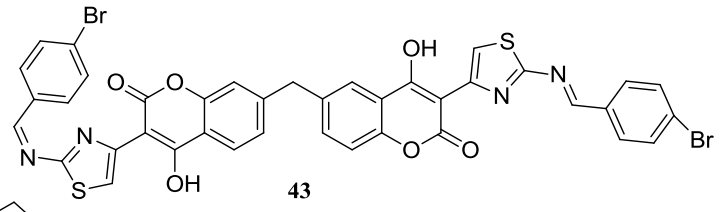

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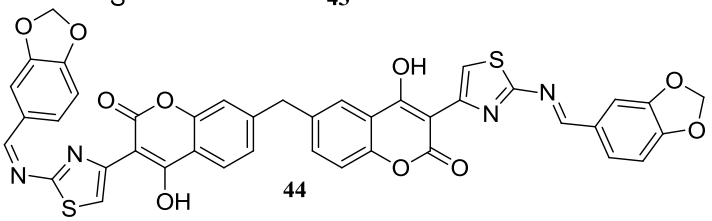

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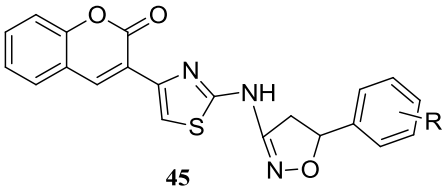
A detailed antifungal study of bis-thiazolyl coumarins was conducted by Raghu and co-workers (2009). Among all the synthesized compounds, **43** and **44** displayed notable activity against most of the tested strains and found to have potent antifungal effect against *Candida albicans*, *Aspergillus fumigates*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

In addition, 5-(4-hydroxy-3-methoxyphenyl)-3-[(4-coumarin-3-yl)-thiazole-2-yl)]-2-iso xazolines (**45**) were also reported to exhibit good to excellent activity against Gram-negative bacterial strains (Desai *et al.*, 2008).

Very recently, Chimenti and co-workers (2009, 2010) studied the anti-*Helicobacter pylori* activity of 4-(coumarin-3-yl)thiazol-2-ylhydrazone derivatives against 20 isolates of *H. pylori*. Only three compounds **46**, **47** and **48** bearing specific heterocyclic rings furan, pyridine and naphthalene respectively on the hydrazone moiety exhibited MIC values (8 µg/mL) comparable to that of the reference drug against some clinical strains of *H. pylori*.



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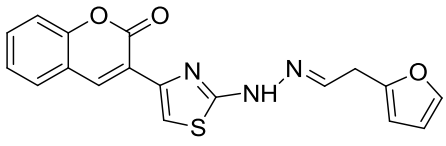

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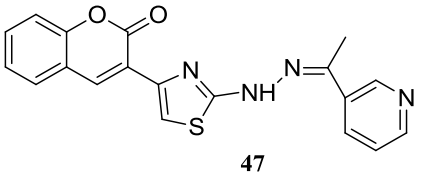

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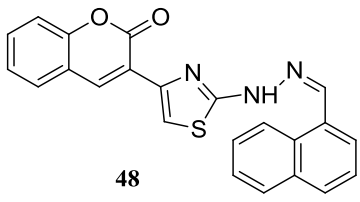

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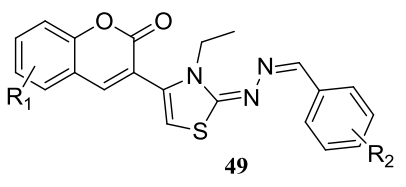
1.3.2.2. Antituberculosis activity of thiazolyl coumarin

Some studies have been conducted on the anti-tuberculosis activity of hydrazinyl thiazolyl coumarins. Karali and co-workers (2002) synthesized a series of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazoline-2-onebenzylidenehydrazones (**49**) via the reaction of 3-bromoacetylcoumarins with 1-substitutedbenzylidene-4-cyclohexyl thiosemicarbazides. All the synthesized derivatives were screened for anti-tuberculosis activity against *M. tuberculosis* H37Rv and found to inhibit the growth of this strain to varying extent. Later, the same group of researchers (Gursoy and Karali, 2003) reported the synthesis and anti-tuberculosis activity of 4-(3-coumarinyl)-3-benzyl-4-thiazoline-2-one benzylidenehydrazones (**50**).

Moreover, the synthesis and anti-tubercular activities of acetyl derivatives of 3-(2-hydroxybenzalhydrazino-4-thiazolyl)coumarins (**51**) obtained by treating them with acetic anhydride in the presence of a catalytic amount of pyridine were also explored (Rao and Reddy, 2003).



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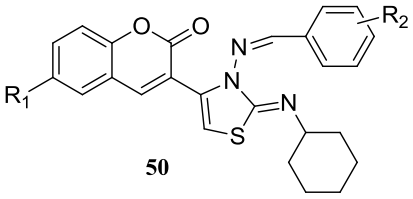

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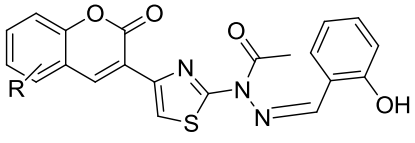

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1.4. Research Objectives

On the basis of all above mentioned evidence, we set out to prepare the new series of biologically active agents containing both important pharmacophores, coumarin and thiazole rings within the molecular framework.

The present work focuses on four main research objectives:

I. To synthesize the new series of heterocyclic compounds by incorporating thiazole ring with coumarin nucleus.

II. To characterise all the synthetic compounds by different spectroscopic techniques (IR, 1D & 2D NMR, HRESIMS) and elemental analysis. To establish the exact configuration of some derivatives by X-ray crystallography.

III. To evaluate antimicrobial activities of the synthesized compounds against Gram-positive (*S. aureus* and *S. Pyogenes*) and Gram-negative (*H. influenzae*) bacteria including *Mycobacterium tuberculosis* as well as against *Candida albicans* (a fungal strain).

IV. To evaluate the antioxidant activities of all the synthesized compounds by using DPPH radical assay and to compare their antioxidant activities to that of a synthetic antioxidant, butylated hydroxytoluene and two natural antioxidants, quercetin and catechin.

CHAPTER TWO

EXPERIMENTAL

2.1. Chemicals and Solvents

The commercial chemicals and reagents used in the syntheses and characterization of all the synthesized compounds are as follows: Acetic acid glacial, AR grade (QRëC); Acetone, AR grade (QRëC); Acetophenone, 99% (Sigma-Aldrich, USA); Ammonium hydroxide, AR grade, 28–30% NH_3, (Sigma-Aldrich, USA); Benzophenone, 99% (Sigma-Aldrich, USA); Bromine (Merck, Germany); 2-Bromobenzaldehyde, (Acros Organics, Belgium); 4-Bromobenzaldehyde, (Acros Organics, Belgium); 5-Bromo,2-hydroxybenzaldehyde, (Acros Organics, Belgium); 4-Butylbenzaldehyde, (Acros Organics, Belgium); Calcium chloride, anhydrous, granular (R & M Chemicals, UK); 2-Chlorobenzaldehyde, (Acros Organics, Belgium); 4-Chlorobenzaldehyde, (Acros Organics, Belgium); Chloroform, AR grade (QRëC); Dichloromethane, AR grade (QRëC); Diethyl ether, AR grade (Lab-Scan, Thailand); Dimethyl sulfoxide-d_6 for NMR (Sigma-Aldrich, USA); 2,4-Dichlorobenzaldehyde, (Acros Organics, Belgium); 2,4-Dihydroxybenzaldehyde, (Acros Organics, Belgium); Ethanol, 99.7%, denatured, AR grade (QRëC); Ethyl acetate, AR grade (Fisher Scientific, UK); Ethyl acetoacetate (Sigma-Aldrich, USA); 2-Fluorobenzaldehyde, (Acros Organics, Belgium); 4-Fluorobenzaldehyde, (Acros Organics, Belgium); 2-Hydroxyacetophenone, (Acros Organics, Belgium); 3-Hydroxyacetophenone, (Acros Organics, Belgium); 4-Hydroxyacetophenone, (Acros Organics, Belgium); 2-Hydroxybenzaldehyde, (Acros Organics, Belgium); 3-Hydroxybenzaldehyde, (Acros Organics, Belgium);

4-Hydroxybenzaldehyde, (Acros Organics, Belgium); 2-Hydroxy,1-naphthaldehyde; Methanol, AR grade (QRëC); 2-Methoxybenzaldehyde, (Acros Organics, Belgium); 4-Methoxybenzaldehyde, (Acros Organics, Belgium); 2-Methylbenzaldehyde, (Acros Organics, Belgium); 4-Methylbenzaldehyde, (Acros Organics, Belgium); Molecular sieves type 4A, beads (Fluka Switzerland); 1-Naphthaldehyde (Aldrich, USA); 3-Nitrobenzaldehyde, (Acros Organics, Belgium); Piperidine (BDH, England); Pyridine (Aldrich, USA); Potassium bromide, FT-IR grade, (Sigma-Aldrich, USA); Salicylaldehyde (Aldrich, USA); TLC silica gel 60 F254, aluminium sheets, 20cm x 20cm (Merck, Germany); Thiosemicarbazide, 99% (Aldrich, USA); *o*-Vanillin (Aldrich, USA). All chemicals and solvents were of reagent grade. All solvents were used without further purification, unless otherwise stated.

2.2. Purification of solvents

2.2.1. Ethanol free chloroform

Two methods were used to remove ethanol from chloroform.

i. First method involved the use of concentrated sulphuric acid for the purification of chloroform. Concentrated sulphuric acid was added to the chloroform and the solution was swirled gently in a separating funnel. After removing the acid, chloroform was washed many times with small portions of distilled water, until water after washing had neutral pH. The chloroform was then dried over anhydrous calcium chloride for overnight and then distilled. Chloroform purified by this method (Williamson et al., 1995) is reported to contain less than 0.001 % (w/w) of ethanol.

ii. In the second method, molecular sieves (4Å) were used to obtain ethanol-free chloroform. Chloroform was washed four times with distilled water and then dried over the molecular sieves (Cohen et al., 2009).

2.2.2. Dry ethanol

Ethanol was dried by refluxing it with anhydrous calcium oxide (250 g/L) for 6 hours. The mixture was then allowed to stand overnight and then distilled under nitrogen (Armarego and Chai, 2009).

2.3.General Experimental Methods

2.3.1. Reaction Monitoring

The progress of all reactions was monitored by thin layer chromatography (TLC) at regular intervals of 30 minutes. The reaction mixture along with the starting materials was spotted on the TLC plates. The TLC plates were developed by using different solvent systems such as acetone: *n*-hexane, chloroform:*n*-hexane and chloroform: methanol. The appearance of product spot and vanishing of reactants spots on the TLC plates determined the optimum reaction time for each reaction. The TLC plates were visualised either under ultra violet radiation at wavelengths of 254 and 365 nm or by developing the spots in an iodine tank.

2.3.2. Moisture-sensitive Reactions

All moisture sensitive reactions were conducted at normal atmospheric pressure and the reaction flasks were affixed with a calcium chloride guard tube. Prior to a reaction, all glasswares were pre-dried at 120 ^{o}C overnight in an oven and assembled hot. Solvents